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K^+ CHANGES IN THE EXTRACELLULAR SPACE OF THE SPINAL CORD AND THEIR PHYSIOLOGICAL ROLE

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SUMMARY

K^+ accumulates in the intercellular space as a result of neuronal activity. The changes in extracellular K^+ concentration, $\Delta[K]_e$ (estimated by K^+-selective microelectrodes), depends on neuronal activity, on the density of discharging neurones and the removal of the accumulated K^+ by diffusion, active transport and current flow through cells. In the mammalian as well as the amphibian spinal cord a single volley in a peripheral nerve increases $[K]_e$ by 0·2–0·5 mmol.l^{-1}, while tetanic stimulation (100 Hz) by 7–8 mmol.l^{-1}, with a maximum in the lower dorsal horn. Increased $[K]_e$ was also found in lumbar segments when the somatosensory cortex of the cat and medulla of the frog were stimulated. In the frog spinal cord, the tactile stimulation of the hindlimb evoked $\Delta[K]_e$ by about 0·1 mmol.l^{-1}, nociceptive stimulation by 0·2–1·0 mmol.l^{-1}. Spontaneous $\Delta[K]_e$ and dorsal root potentials (DRPs) were observed at various intervals after stimulation, associated with the decay phase of $\Delta[K]_e$.

It was shown that primary afferent depolarization (PAD) consists of two components: the 'early' component (mediated by GABA and depressed by picrotoxin or bicuculline) and the 'late' K^+ component (potentiated by picrotoxin and bicuculline). Even when increased $[K]_e$ produces PAD, this does not mean that it also results in presynaptic inhibition. It was found that the $\Delta[K]_e$ produced depolarization of motoneurones and neuroglia and there is every reason to believe that this also applies to the interneurones. Evidence is available that an increase of $[K]_e$ up to 6 mmol.l^{-1} facilitates impulse transmission in the spinal cord while higher levels result in its inhibition.

INTRODUCTION

In the last decade the idea developed that the ionic changes in the narrow intercellular space resulting from neuronal activity could serve as a way of transmitting information between the tightly packed neurones or between neurones and glia. The development of ion-selective microelectrodes in 1971 by Walker has enabled direct measurements of rapidly changing ionic concentrations. The application of this method to the study of K^+ changes in extracellular space ($\Delta[K]_e$) of CNS has elucidated the question of the $\Delta[K]_e$ occurring in the nervous tissue under normal and pathological conditions and where these $\Delta[K]_e$ take place. Several suggestions have been made and have received experimental support as to the manner in which these $\Delta[K]_e$ can affect the function of the CNS (for review see Somjen, 1979; Nicholson,

1980; Hník *et al.* 1980; Syková, Hník & Vyklický, 1981). It is now possible to indicate some of the ways in which $\Delta[K]_e$ can modulate impulse activity. A large number of these experiments were performed on the spinal cord of mammals as well as of amphibians.

K^+ ACCUMULATION IN THE EXTRACELLULAR SPACE OF THE SPINAL CORD

In the first experiments with K^+ selective microelectrodes performed in the rat spinal cord in 1972, it was found that $[K]_e$ increased by several mmol.l^{-1} during tetanic stimulation of the peripheral nerve (Vyklický *et al.* 1972; Krnjević & Morris, 1972). Since then a large number of reports have confirmed this finding and detailed studies of K^+ transient changes have been made in the mammalian as well as in the amphibian spinal cord (Ten Bruggencate, Lux & Liebel, 1974; Kříž *et al.* 1974; Somjen & Lothman, 1974; Kříž *et al.* 1975; Krnjević & Morris, 1975*a*, *b*; Lothman & Somjen, 1975; Vyklický, Syková & Kříž, 1975; Syková *et al.* 1976; Syková & Vyklický, 1977; Syková & Vyklický, 1978; Nicoll, 1978; Syková, Czéh & Kříž, 1980*a*). It was shown that K^+ accumulates preferentially in deeper layers of the dorsal horn and in the intermediate region of the mammalian as well as the amphibian spinal cord following either single or tetanic electrical stimulation of peripheral nerves (Fig. 1), or adequate stimulation of the skin. In this region, many primary afferents terminate and neuronal density is very high – about six neurones in 100 μm^3 (Aitken & Bridger, 1961; Kříž *et al.* 1974; Székely, 1976). K^+ are released from active neurones as well as from primary afferent fibres (Syková & Vyklický, 1977). The $\Delta[K]_e$ in the upper dorsal horn or in the ventral horn following peripheral stimulation are negligible. The increased $[K]_e$ was also found in lumbar segments on electrical stimulation of the somatosensory cortex of the cat and the caudal part of the medulla of the frog (Syková, Kříž & Vyklický, 1975; Syková, Kříž & Czéh, 1980*b*).

The accumulation of K^+ depends on the frequency and duration of stimulation. Its maximum at 100 Hz stimulation is usually reached after 10–15 s and does not exceed 10 mmol.l^{-1} (Fig. 2). Stimulation at higher frequencies or of longer duration caused no further increase in $[K]_e$. However, even stimulation at 3 Hz increased $[K]_e$ by about 1 mmol.l^{-1} (Kříž *et al.* 1975). K^+ accumulation of up to 1 mmol.l^{-1} was also found under more physiological conditions, i.e. during adequate stimulation of skin nociceptors (Syková *et al.* 1980*a*).

Tetanic stimulation of a peripheral nerve produces potential shifts in the spinal cord (Fig. 2), which were recorded with extracellularly placed microelectrodes or with the reference barrel of double-barrel K^+-selective microelectrodes (Somjen, 1969; Somjen, 1970; Kříž *et al.* 1975). In the mammalian spinal cord these shifts roughly reflect glial cell depolarization as far as the amplitude, time course and spatial distribution is concerned. Correlation also exists with the distribution of $\Delta[K]_e$ (Somjen & Lothman, 1974; Lothman & Somjen, 1975).

The clearance of accumulated K^+ from the extracellular space

The dynamics of $\Delta[K]_e$ are the result of passive and active transport across the cell membrane, diffusion in the intercellular space, voltage gradients in the tissue, dispersal across the blood–brain barrier and of water movements between active and

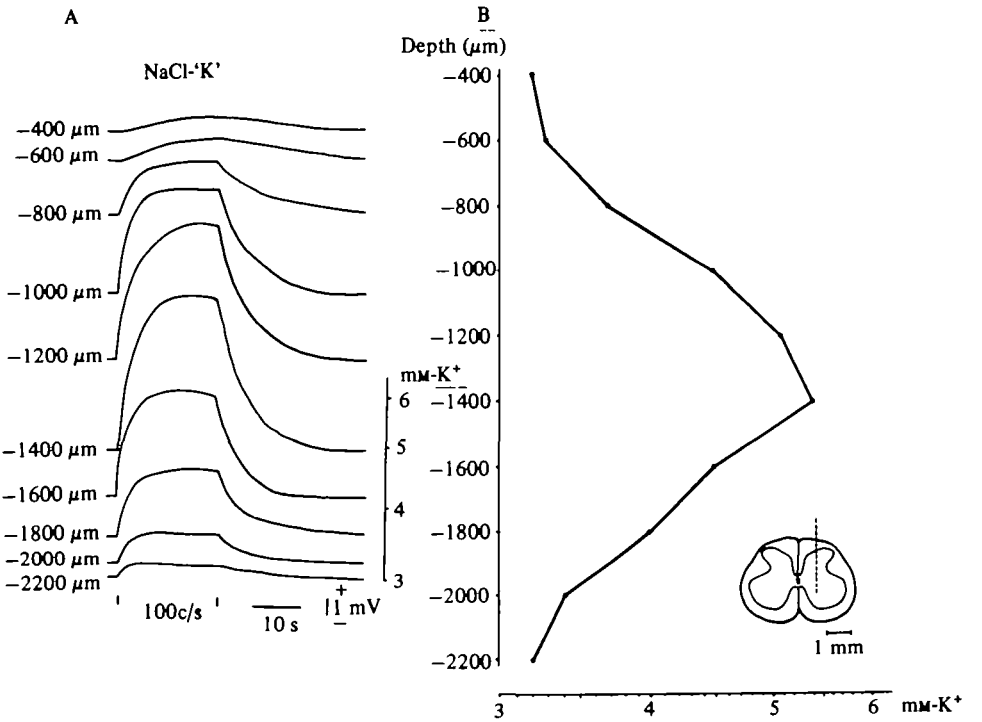


Fig. 1. Changes in $[K]_o$ produced by 100 Hz stimulation of the posterior tibial nerve at various depths in the L_7 spinal segment of the cat. (A) The depth of electrode insertion is indicated for each record. The vertical bars below records indicate the period of stimulation. In diagram B the depth is indicated on the ordinate and increase in $[K]_o$ on the abscissa. (From Křiž *et al.* 1974.)

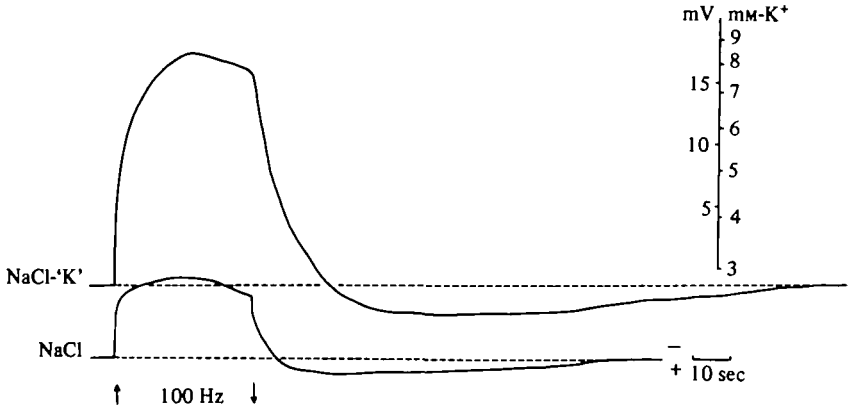


Fig. 2. Changes in $[K]_o$ produced by tetanic stimulation of posterior tibial nerve. Upper record (NaCl-'K') represents the time course of the changes in $[K]_o$ evoked by 100 Hz stimulation lasting 35 s. Increase in K^+ activity from 3 to 9 mmol.l^{-1} is followed by long-lasting subnormal level of $[K]_o$. The lower record (NaCl) represents the corresponding negative potential shift obtained by NaCl channel against ground followed by a long-lasting positivity. (From Křiž *et al.* 1975.)

passive areas (for review see Nicholson, 1980; Syková *et al.* 1981). In our experiments the level of increased $[K]_e$ did not persist throughout the whole period of higher-frequency tetanic stimulation, but declined after its maximum had been reached (Fig. 2). Furthermore, the process of redistribution of accumulated K^+ in the mammalian spinal cord does not cease after the original resting level is reached, but $[K]_e$ continues to decrease below the initial resting level. This decrease of $[K]_e$ during stimulation as well as the 'undershoot' apparently reflect the active processes which participate in the redistribution of accumulated K^+. The undershoot was only found to be present in the close vicinity of discharging neurones in the lower dorsal horn not depending on the absolute level of $\Delta[K]_e$. Because there are no data in favour of the idea that the glia is specialized and not dispersed uniformly, it is likely that mainly neuronal elements and not glial cells are responsible for the active reabsorption of accumulated K^+ (Kříž *et al.* 1975).

The metabolic character of the undershoot was demonstrated by its high sensitivity to oxygen supply and body temperature. In the cat, when the respiration was impaired and blood pressure fell under 100–120 torr the undershoot disappeared. The active processes of replacing accumulated K^+ are, however, still able to bring it to the resting level (3 $mmol.l^{-1}$). When the blood pressure had fallen to 80 torr or lower, the clearance of K^+ from extracellular space became incomplete and the $[K]_e$ was gradually built up (Kříž *et al.* 1975). Barbiturate anaesthesia in therapeutic doses slowed the rate of decay of stimulation-evoked $\Delta[K]_e$ and no undershoot was found.

In the frog spinal cord, the depolarization of dorsal root fibres in response to electrical as well as adequate stimulation was followed by their hyperpolarization (see Fig. 15). The amplitude and duration of hyperpolarization correlated with the magnitude and time course of elevated $[K]_e$. The dorsal root hyperpolarization (DRH) was highly sensitive to anoxia and was eliminated by the application of ouabain and by the replacement of Na^+ in the Ringer solution by Li^+ (Davidoff & Hackman, 1980; Syková, unpublished observations). There are good reasons to believe that the undershoot and DRH are the result of active K^+ uptake by stimulation of the Na–K pump in neurones as well as in primary afferent fibres themselves (Kříž *et al.* 1975; Davidoff & Hackman, 1980; Syková *et al.* 1980*b*). Active re-uptake may in fact be stimulated by the rise in $[K]_e$ (passive elements) as well as by the rise in $[Na]_i$ (active elements). Thus, the Na–K pump is probably the main mechanism for K^+ clearance and a substantially smaller amount of K^+ released to circumscribed area escapes this reabsorption by following its gradient in the intercellular space or by being conveyed by glial cells. The passive redistribution of accumulated K^+ could contribute more under some pathological states when the active processes are blocked but further information would be worthwhile.

THE ROLE OF K^+ ACCUMULATION IN PRIMARY AFFERENT DEPOLARIZATION

The finding that K^+ accumulation is very circumscribed, with a spatial maximum as well as a rapid time course in deeper layers of the dorsal horn, has led to the revival and reinvestigation of the original hypothesis of Barron & Matthews (1938). They

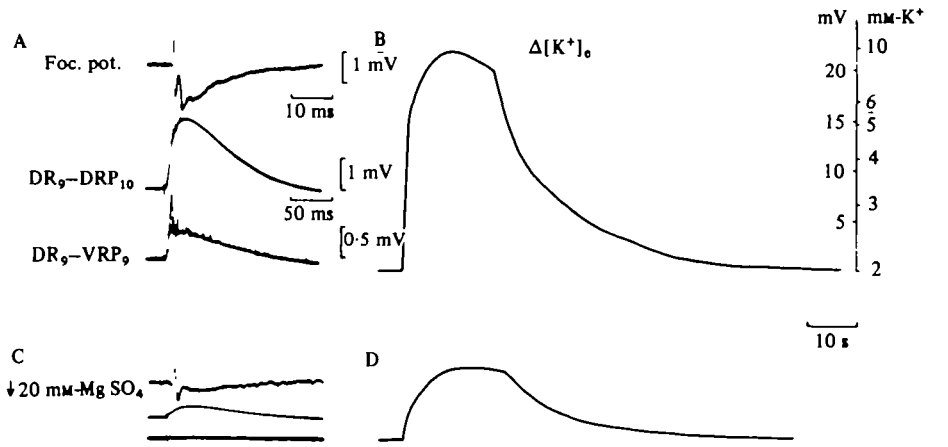


Fig. 3. Effect of high Mg^{2+} concentration on dorsal-root potentials (DR-DRPs), ventral root potentials (DR-VRPs), focal potentials and transient changes in $[K]_e$ evoked in isolated spinal cord of frog by orthodromic stimulation. Focal potentials were evoked by single stimuli in DR_9 and recorded by a reference channel of the double-barrel potassium-sensitive microelectrode against ground. The microelectrode was introduced in the intermediate region of the IXth lumbar segment. Increase in $[K]_e$ was induced by 100 Hz stimulation of DR_9. (A, B) Control records; (C, D) 20 mmol.l^{-1} $MgSO_4$ was added to Ringer solution. The time scale 50 ms relates to DRPs and VRPs. The calibration on the right indicates changes in K^+ in Ringer solution corresponding to deflection in mV. (From Syková & Vyklický, 1977.)

suggested that depolarization of primary afferent terminals which were recorded as slow positive surface potentials, i.e. cord dorsum potentials (Gasser & Graham, 1933) or negative dorsal root potentials (DRPs) (Barron & Matthews, 1938; Eccles & Malcolm, 1946), could be explained by changes in the ionic concentrations in synaptic clefts around active neurones and unmyelinated nerve terminals. It was later postulated that primary afferent depolarization (PAD) was the mechanism underlying presynaptic inhibition (Eccles, Eccles & Magni, 1961) and that changes in the ionic permeability of primary afferent terminals were produced by the action of a specific depolarizing transmitter – GABA, released at axo-axonic synapses (Schmidt, 1971; Levy, 1977).

Recent findings have led to the conclusion that PAD has at least two components which have an entirely different mechanism (Barker, Nicoll & Padjen, 1975; Syková & Vyklický, 1978; Davidoff, Hackman & Osorio, 1980; Syková *et al.* 1980*a*). The first, 'early' component with the shorter latency, results from the action of a specific transmitter, probably GABA. The greater part of DRPs evoked by single electrical shocks can be attributed to this mechanism. The second, 'late' and long-lasting component of PAD with the slower rise time is caused by K^+ accumulation in the dorsal horn. This component increases after repetitive electrical stimulation or prolonged adequate stimulation, especially that of skin nociceptors (Syková & Vyklický, 1977; Nicoll, 1979; Davidoff *et al.* 1980; Syková *et al.* 1980*a*).

This so-called 'dual' mechanism of the origin of the DRPs was supported by experiments on the isolated spinal cord of frog. The experiments demonstrated that

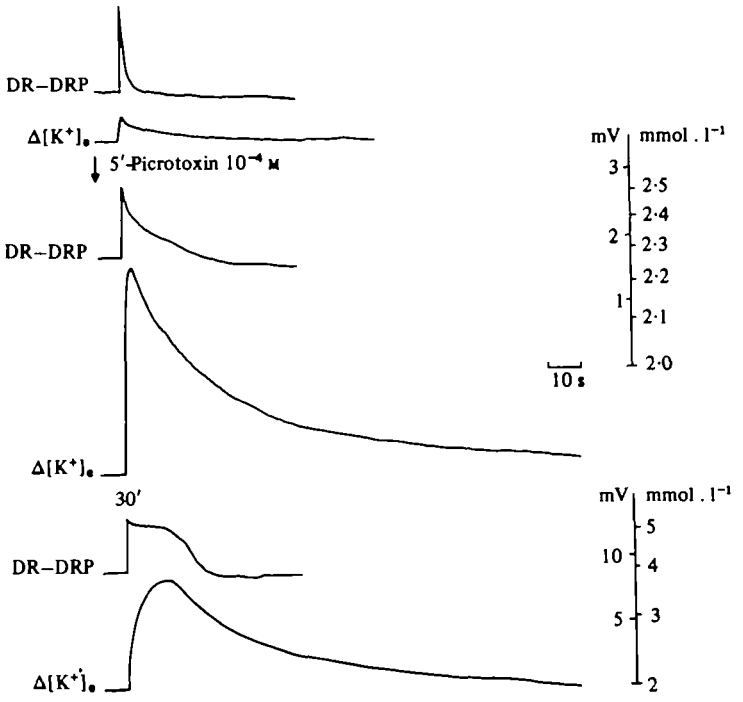


Fig. 4. The effects of picrotoxin (10^{-4} mol.l^{-1}) on the dorsal root potentials (DR-DRPs) and changes in $[K]_o$ in the isolated frog spinal cord. Note the prolongation of DRPs 5 s and 30 s after picrotoxin and the corresponding increase in $[K]_o$. Note that the calibration is different for $\Delta[K]_o$ after 30 s.

increased excitability of primary afferents (Wall, 1958) and DRPs evoked by stimulation of the adjacent dorsal root (DR-DRPs) could be elicited by direct electrical stimulation of the cord when all synaptic transmission was blocked by adding 20 mmol.l^{-1} $MgSO_4$ to the Ringer solution (Vyklický, Syková & Mellerová, 1976). The ventral root discharge and focal potentials evoked by a single volley in the dorsal root disappeared, but the DR-DRPs were not eliminated (Syková & Vyklický, 1977). The DR-DRPs diminished in amplitude to about 10% of the control and the K^+ transients, evoked by 100 Hz stimulation also, decreased to about 10–15% of their original values (Fig. 3). The Mg^{2+} resistant DR-DRPs have been called 'asynaptic' and the most likely explanation of their origin is the depolarization by K^+ released from the stimulated primary afferent fibres. They were found to exhibit non-linear summation (Syková & Vyklický, 1977; Nicoll, 1979), similar to that occurring in glial cells during repetitive stimulation (Kuffler, Nicholls & Orkand, 1966). It is reasonable to assume that the K^+ released from the postsynaptic neurones during their activation also significantly contributes to PAD when synaptic transmission is intact and that the K^+ component in normal DR-DRPs evoked by a single afferent volley should therefore be higher than 10%.

Further evidence for the fact that DRPs have a K^+ component was the finding that only the 'early' component of the DRPs with the shorter latency is depressed by the GABA-antagonists, picrotoxin and bicuculline (Barker *et al.* 1975; Lothman &

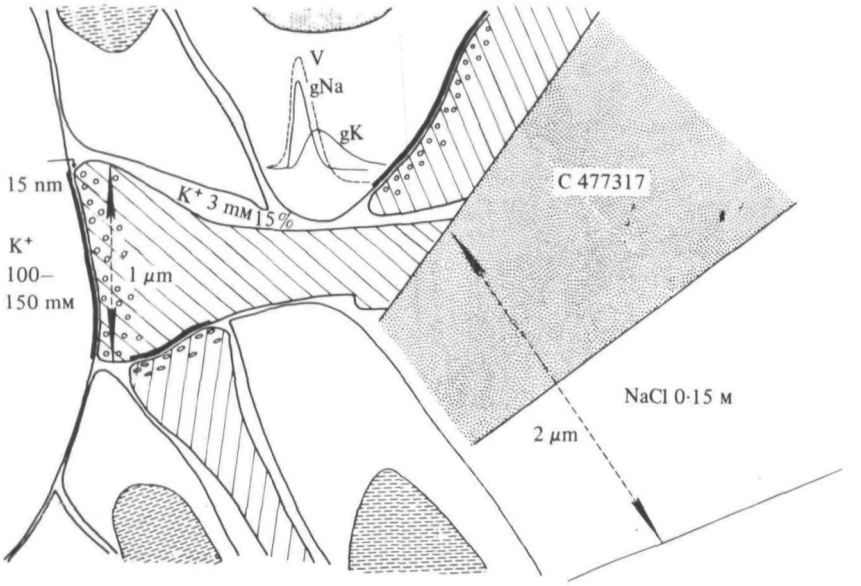


Fig. 5. Scheme of the experimental situation, when measuring $\Delta[K]_o$ with double-barrel potassium selective microelectrodes with the tip about 2 μm. The synaptic cleft between primary afferent (diameter about 1 μm) and secondary neurone is 15 nm. Intercellular K^+ concentration 100–150 mmol.l^{-1}, extracellular K^+ concentration 3 mmol.l^{-1}.

Somjen, 1976; Syková & Vyklický, 1978; Davidoff *et al.* 1980). The long-lasting 'late' component of the DRPs is augmented and accompanied by a substantial increase in $[K]_e$ resulting from greatly enhanced neuronal firing. As demonstrated in Fig. 4, picrotoxin in concentrations 10^{-4} mol.l^{-1} depressed the 'early' component of DR-DRPs to about 60% of the control, but it prolonged and enhanced the 'late' phase of DR-DRPs and increased $\Delta[K]_e$ from 0·05 mmol.l^{-1} to 4 mmol.l^{-1}. It is evident that the duration and amplitude of the 'late' DRPs corresponds to the increase in $[K]_e$.

The shift in the time course of $[K]_e$ changes, when compared with that of evoked DRPs, is apparently due to the fact that the diffusion time for K^+ released from the secondary neurones to reach primary afferent terminals will be much shorter than that necessary for reaching the tip of the microelectrode. The primary afferent terminals are separated from secondary neurones only by a narrow cleft of about 15 nm width, while the K^+-selective microelectrode with a 2–4 μm tip diameter cannot be placed anywhere as close to the surface of a neurone (Fig. 5). The K^+-selective microelectrode therefore reflects changes of $[K]_e$ in a relatively large volume of intercellular space to which many neurones and fibres contribute by releasing K^+ during their spike activity (Neher & Lux, 1973; Kříž *et al.* 1975).

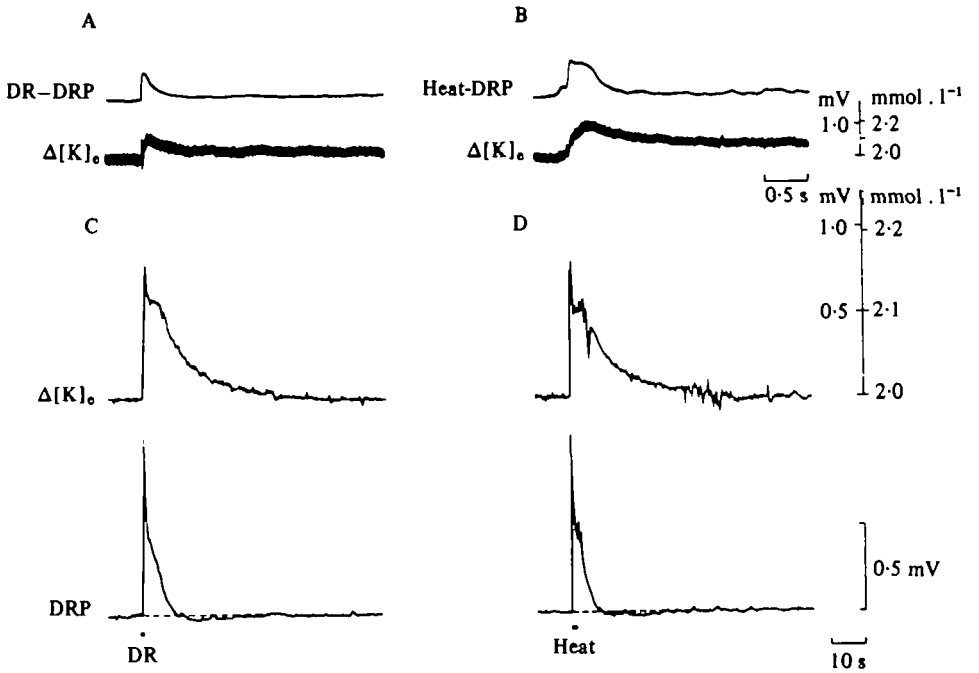


Fig. 6. Dorsal root potentials (DR-DRPs) and $\Delta[K]_e$ in spinal cord of the frog evoked by stimulation of the skin of the hindlimb. (A, C) Responses to single volley applied to the skin. (B, D) Responses to stimulation with hot water applied for 1–2 s (60 °C). (A, B) Oscilloscopic recordings from spinal cord *in situ*. (C, D) Responses from experiment on isolated spinal cord–hindlimb preparation recorded on a two-channel pen writer (LKB).

K^+ component in DRPs evoked by nociceptive stimulation

The 'late', presumably K^+ component, was found even in normal DRPs evoked by single supramaximal shocks applied to the skin of the hindlimb and especially in those evoked by nociceptive stimulation (Fig. 6). The 'late' component with the slower time course may be recognized as a hump on the decay phase of these DRPs. When the activity of dorsal horn neurones in the frog was studied in response to nociceptive stimulation of the skin with hot water (50–60 °C applied for 1–2 s) it was found to produce long-lasting, high-frequency repetitive firing (0·5–2 s; 50–200 Hz) in neurones in the dorsal horn and intermediate region of the cord. A single volley applied to the skin usually evoked only 2–5 spikes (Czéh, Syková & Vyklický, 1980). Thus, the usual 'late' component of DRPs evoked by nociceptive stimulation could be produced by greater K^+ accumulation arising from long-lasting firing leading to a greater and longer increase in $[K]_e$, similarly to what had previously been described as causing prolonged DRPs after application of the picrotoxin or sustained DRPs during tetanic electrical stimulation (Lothman & Somjen, 1975; Syková & Vyklický, 1978; Nicoll, 1979; Davidoff *et al.* 1980; Syková *et al.* 1980*a*, *b*). It was assumed that the K^+ component in DRPs gradually increased with the frequency and duration of stimulation and could represent about 90% of their size. The $\Delta[K]_e$ produced by the nociceptive stimulation can thus be compared with those evoked by tetanic stimulation rather than those evoked by a single volley and the K^+ component could be of greater physiological significance than has hitherto been believed.

FUNCTIONAL SIGNIFICANCE OF INCREASED $[K]_e$ – ITS EFFECT ON SYNAPTIC TRANSMISSION

The extent to which the increase of $[K]_e$ could affect neuronal function has been discussed for a long time, and several mechanisms of its action have been suggested. It was assumed that $\Delta[K]_e$ reduces transmitter release by curtailing the presynaptic spike amplitude by presynaptic depolarization. On the other hand, in the absence of impulse activity, the direct depolarization by raised K^+ evokes transmitter release (Liley, 1956; Eccles, 1964; Gage & Quastel, 1965). This depolarization-induced transmitter release can furthermore diminish evoked transmitter release (Hagiwara & Tasaki, 1958; Erulkar & Weight, 1977). Moreover, these effects of raised $[K]_e$ could be enhanced by concomitantly decreasing $[Ca^{2+}]_e$ (Somjen, 1979; Somjen, 1980). Both these ionic changes would affect the transmitter release in the same direction.

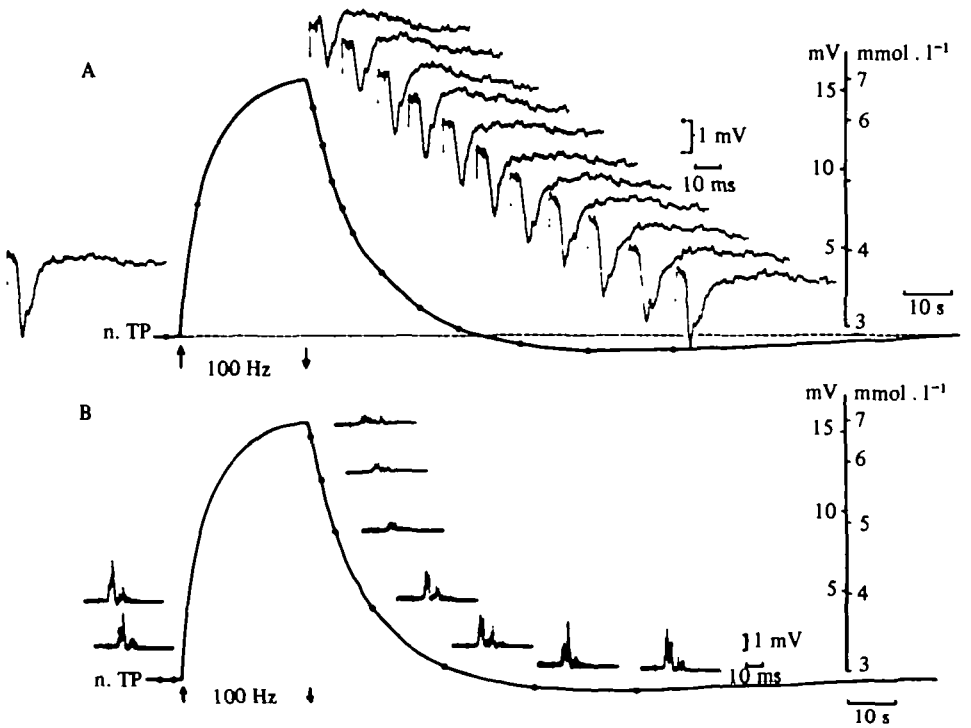
Instead of its presynaptic action, the increased $[K]_e$ could influence spinal cord transmission by depolarization of neurones and glial cells. Especially at low levels, the K^+-mediated neuronal depolarization will enhance neuronal excitability, which may dominate over its presynaptic effect.

Spinal cord transmission and $\Delta[K]_e$ evoked by repetitive stimulation

To examine the effects of extracellular K^+ accumulation resulting from prior activity, the electrophysiological changes in spinal cord transmission were recorded in the spinal cord of the cat as well as in the isolated frog spinal cord. It was found that changes in impulse transmission correlate with the accumulation of K^+ in the extracellular space. In the cat cord the changes in the amplitude of the field potentials and ventral root discharges at various levels of $[K]_e$ were examined. It can be seen from Fig. 7 that both were depressed during the phase of $[K]_e$ elevation. The correlation between the depression of DR-DRPs and the K^+ elevation after tetanic stimulation was shown in experiments on the frog isolated spinal cord (see Fig. 8).

$\Delta[K]_e$ and membrane potential of neurones and glial cells

In the isolated spinal cord of the frog many of the after-effects of tetanic dorsal root stimulation, which evoked the large $\Delta[K]_e$, could be mimicked by increasing [K] in perfusion fluid. These include the depolarization of neurones and neuroglia. Intracellular recordings from motoneurones and glial cells revealed that they were depolarized both by increasing [K] in perfusing fluid and by tetanic supramaximal stimulation of the dorsal root (Figs. 9, 10). Maximum depolarization of motoneurones in response to a dorsal root tetanus (100 Hz, 15–20 s) was about 35 mV. This response was equivalent to the effect of 20 mmol.l^{-1} K^+ in the perfusing fluid. Surprisingly, the maximum glial depolarization to stimulation was only 10–12 mV, which is the equivalent of adding about 10 mmol.l^{-1} K^+ to the bath. The glial depolarization was not affected by their localization at different depths, even when it was found, using K^+-selective microelectrodes, that there is a highly non-uniform distribution of K^+ accumulation following dorsal root tetanic stimulation (see Fig. 8, and Syková *et al.* 1976). These results suggest that glial cells in the amphibian spinal cord may form an electrical syncytium (Syková & Orkand, 1980). The glial cells are accurate K^+ electrodes only when entirely surrounded by uniform $[K]_e$ (Orkand, Nicholls &



(Fig. 7. (A) The effect of accumulated K^+_e in the spinal cord of the cat on the amplitude of the focal potentials in L_7 segment. $\Delta[K]_e$ induced by stimulation of the posterior tibial nerve at 100 Hz. The focal potentials were evoked by a single volley in the same nerve at intervals shown by points on the curve of $\Delta[K]_e$. (B) The effect of accumulated K^+ on the ventral root discharge. The ventral root discharge was evoked by single volley in the same nerve at intervals pointed at the record of $\Delta[K]_e$ (From Kříž *et al.* 1975.)

Kuffler, 1966) and their membrane potential indicates only an average K^+ when the K^+ differs in various parts of the glial syncytium in the cord. On the other hand, the estimated increase in $[K]_e$ up to about 20 mmol.l^{-1} obtained from a comparison of the bath concentration needed to depolarize a motoneurone to the same level as found 1 s after dorsal root tetanization is about twice the increase estimated from measurements with K^+-selective microelectrodes (Syková *et al.* 1976) or glial depolarization (Nicoll, 1979; Syková & Orkand, 1980). Instead of the possible underestimation of $\Delta[K]_e$ in measurements with K^+-selective microelectrodes (see p. 99 and Fig. 5) there is a possibility that released amino acids, polypeptides, local changes in pH or even anoxia contributed to the observed changes.

The effects of $\Delta[K]_e$ on synaptic potentials and spontaneous activity

There is a parallel depression of motoneurone spike and EPSP amplitude with depression of DRPs and depolarization of motoneurones in the frog spinal cord produced by increased $[K]_e$ in the perfusion solution or by tetanic dorsal root stimulation. All these potentials return to control values after washing out the increased $[K]_e$ or after its redistribution (Fig. 11; 12). The correlation of the changes in all thes

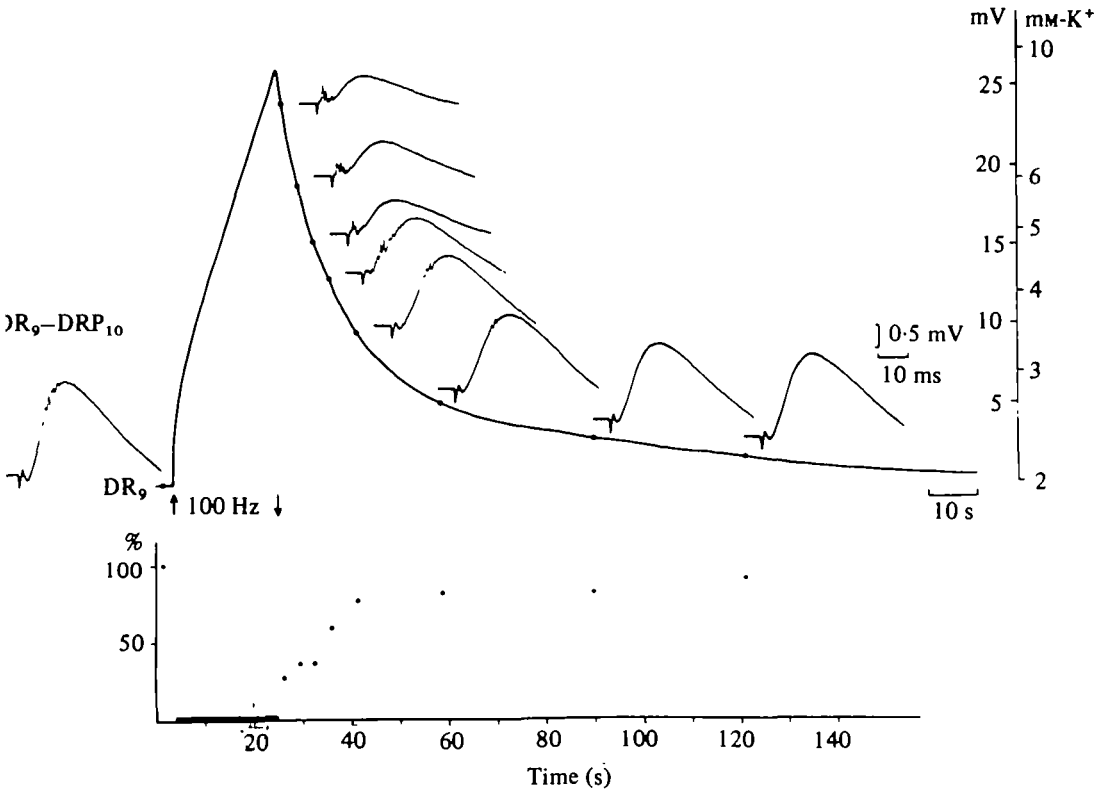


Fig. 8. Depression of DRPs associated with increased $[K]_o$ induced in the IXth segment by stimulation of DR_9 at 100 Hz. Full circles in the record of changes in $[K]_o$ indicate when $DRPs_{10}$ were recorded in response to a single volley in DR_9. The stimulation of DR_9 at 100 Hz lasted 20 s and is indicated by arrows and a thick line in the diagram. The amplitudes of $DRPs_{10}$ were plotted as percentage of the control at various intervals after the end of DR_9 stimulation. (From Syková *et al.* 1976).

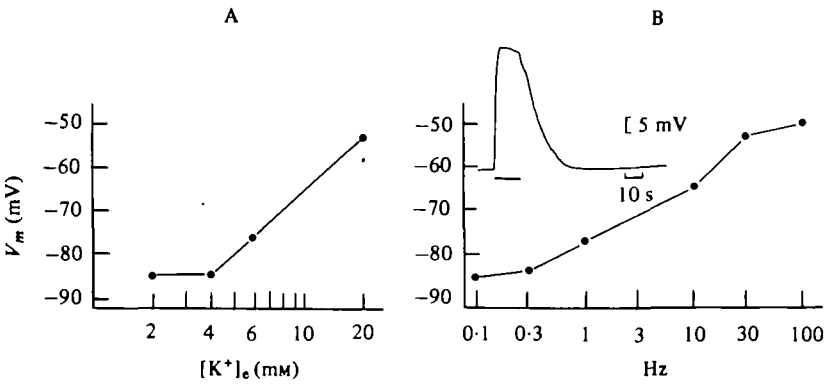


Fig. 9. Effect of $[K]_o$ and dorsal root stimulation on membrane potential of a motoneurone. All measurements are from a maintained penetration of a single neurone. (A) Steady-state membrane potential during perfusion of the spinal cord with 2, 4, 6 and 20 mmol.l^{-1}. (B) Membrane potential of the same motoneurone 0·5 s following a 15 s dorsal root tetanus at the frequencies indicated. Inset record obtained with tetanic stimulation of 100 Hz for 15 s. The time constant of a pen recorder was about 0·2 s. (From Syková & Orkand, 1980.)

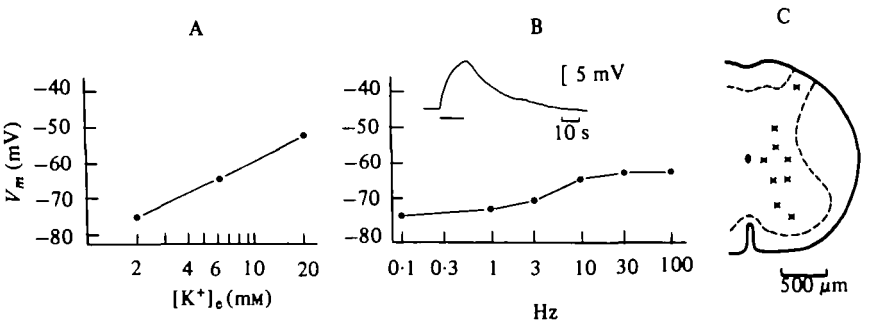


Fig. 10. Effect of $[K]_o$ and orthodromic tetanus on glial cell membrane potential. (A) Relation between $[K]_o$ and membrane potential of a single glial cell. (B) Glial membrane potential at the end of orthodromic tetanic stimulation at indicated frequencies for 15 s (same cell as in A). Inset shows record obtained with stimulation at 100 Hz for 15 s. (C) Distribution of nine glial cell recording sites in spinal cords where maximal tetanic stimulation produced glial cell depolarizations of 10–12 mV. (From Syková & Orkand, 1980.)

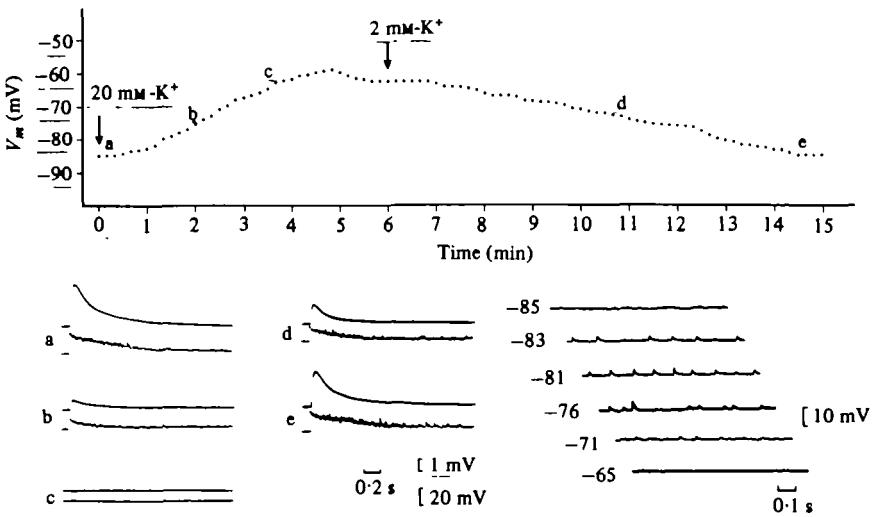


Fig. 11. Effect of elevated $[K]_o$ on dorsal root potentials and motoneurone membrane potential, excitatory postsynaptic potentials and spontaneous synaptic activity. Top: motoneurone resting potential with change in $[K]_o$ from 2 to 20 and back to 2 mmol.l^{-1} (flow rate about 2 ml/min). The membrane was more depolarized about 5 min after changing to 20 mmol.l^{-1} K^+ than at 6 min due to increased neuronal activity resulting from elevated K^+. Bottom: (*a–e*) the upper record is the potential recorded extracellularly from the dorsal root following a single volley in an adjacent dorsal root, the lower record is the intracellularly recorded EPSP produced by the same stimulus. (*a*) Control; (*b*) 2 min after 20 mmol.l^{-1} K^+; (*c*) 6 min after 20 mmol.l^{-1} K^+; (*d*) 4 min after 2 mmol.l^{-1} K^+; (*e*) 7 min after 2 mmol.l^{-1} K^+. Bottom right: records of spontaneous synaptic activity intracellularly from motoneurone at indicated membrane potentials. (From Syková & Orkand, 1980.)

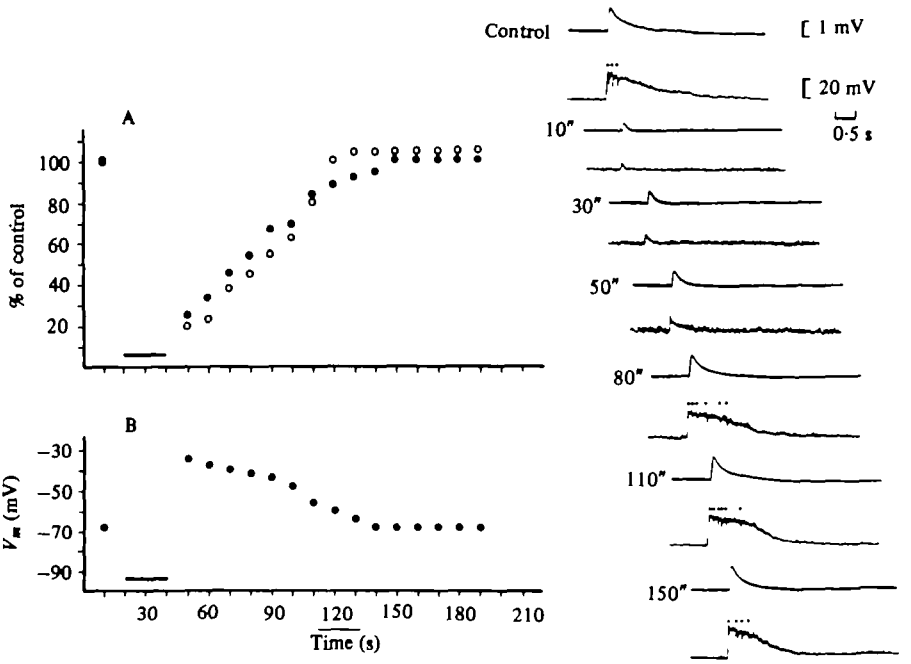


Fig. 12. Inhibition of dorsal root potentials and excitatory postsynaptic potentials following a dorsal root tetanus. (A) Amplitudes of DR-DRP (●) and EPSP (○) after tetanic stimulation of the adjacent dorsal root at 100 Hz for 20 s. (B) Graph of motoneurone resting potential under the same conditions as above. Records are pairs of tracing of DRP (upper) and EPSP (lower) at times after tetanus indicated. Dots above EPSP indicate occurrence of motoneurone spikes which are too brief to be visible at the slow sweep speed. Note prolongation of EPSP and increased numbers of spikes during recovery. (From Syková & Orkand, 1980.)

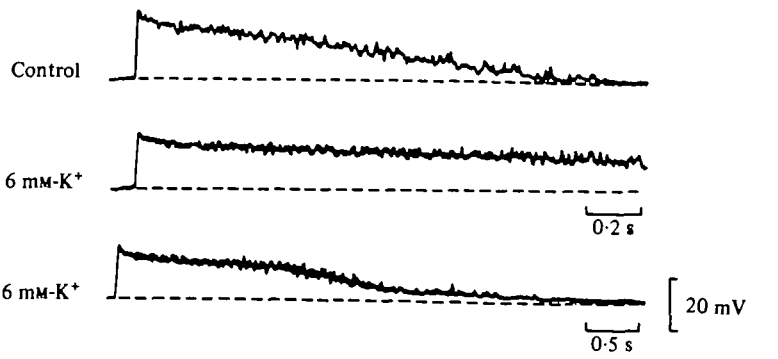


Fig. 13. Prolongation of excitatory postsynaptic potential with raised $[K]_e$. Intracellular recording from a single motoneurone in 2 mmol.l^{-1} K^+ (control) and after elevation of the $[K]_e$ in the perfusion fluid to 6 mmol.l^{-1}. Lowest trace at 2·5 × slower sweep speed. (From Syková & Orkand, 1980.)

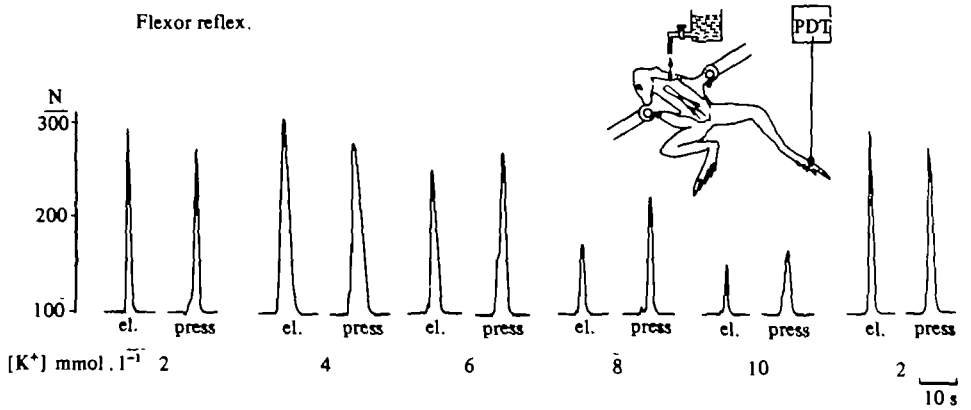


Fig. 14. Flexor reflex responses in the frog during superfusion of the spinal cord with solutions of various concentrations. Diagram: the spinal cord was completely isolated, with the exception of the ventral and dorsal roots IX and X which remained connected with the periphery, and perfused with oxygenated Ringer solution. Flexor reflex was evoked by pinching the skin of the foot with a forceps (press) or with a series of rectangular electrical pulses (el., 13 pulses, 3×10^{-4} s, 20 V at 100 Hz) applied to the skin of the foot by a pair of platinum-wire electrodes. PDT represents a photoelectric displacement transducer with exchangeable springs, which allowed adjustment to various forces. The lever of the transducer was fixed to the interdigital membrane by a thread. The force exerted during flexor reflex is expressed in Newtons (N). Concentration of K^+ in the solution was raised from 2 to 4, 6, 8 and 10 mmol.l^{-1}. Before testing, 30 min were allowed for superfusion with solution containing a higher concentration of K^+ and after testing, 60 min for recovery. (From Vyklický & Syková, 1980.)

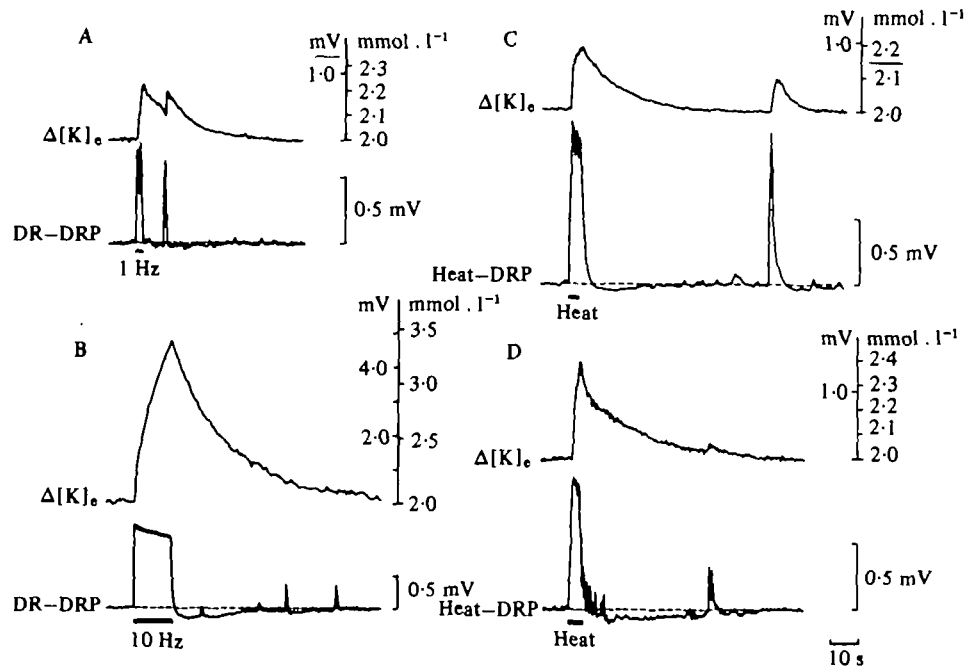


Fig. 15. Evoked and spontaneous changes in $[K]_e$ and dorsal root potentials in IXth spinal segment of the frog. (A, B) Electrical stimulation of the skin, (C, D) nociceptive stimulation with hot water (60 °C) applied to the skin of the hindlimb. (A, B, D) From the experiment on spinal cord *in situ*. (C) From isolated spinal cord–hindlimb preparation.

phenomena suggests that they can be of common origin (i.e. a rise in $[K]_e$). The relatively low increase of $[K]_e$ evoked by tetanic dorsal root stimulation or increasing K^+ in the bath and associated with the smaller changes in membrane potential, led to an increase in spontaneous synaptic activity (Fig. 11, bottom right).

There is sufficient information about the fact that the large increase in $[K]_e$ exceeding 6 mmol.l^{-1} inhibits transmission in the spinal cord (Kříž *et al.* 1975; Krnjević & Morris, 1976; Somjen, 1979; Syková & Orkand, 1980, Vyklický & Syková, 1980), since it causes significant depolarization of membranes (Fig. 9) and alterations in synaptic function. But even small changes in $[K]_e$ should affect the functional properties of neurones (i.e. the threshold, the rate and amount of spontaneous activity and the amount of transmitter release at synapses). In the frog spinal cord, $\Delta[K]_e$ in the range 0·5–6·0 mmol.l^{-1} gave an increase in spontaneous as well as evoked activity (Figs. 11, 12) prolonged motoneuronal EPSPs (Fig. 13) and enhanced reflex activity (Fig. 14). In the experiments with adequate stimulation of the skin, spontaneous DRPs, ventral root potentials and $\Delta[K]_e$ were frequently observed at various intervals after preceding electrical and adequate stimulation, but mainly during the period in which elevated $[K]_e$ was returning to initial levels, as measured by K^+-selective microelectrodes (Fig. 15). This suggests that there occurs a period of increased excitability due to partial depolarization of neurones which enhances their excitability.

CONCLUDING REMARKS

Besides the role of $[K]_e$ changes under pathophysiological conditions, the main question concerns its role in normal nervous function. Recently it was found that K^+ accumulation resulting from prior intraspinal activity does occur even under physiological conditions (i.e. in response to adequate stimulation of the skin). We may predict that every process leading to long-lasting, high-frequency discharges in certain groups of neurones and primary afferent fibres results in K^+ accumulation. The increase in $[K]_e$ measured by K^+-selective microelectrodes (K-ISM) reflects the density of active elements, the intensity of their activity and their distance from the recording microelectrode. It is believed that the changes in $[K]_e$ during prolonged high-frequency stimulation are not seriously underestimated when measured with K-ISMs because of the 'steady state' achieved. However, the situation is less clear during adequate stimulation of the skin (nociceptive, tactile), short electrical low-frequency tetanic stimulation of peripheral nerves or stimulation with single electrical pulses. The dead space created by the microelectrode, destruction of neighbouring cells and fibres necessarily causes an underestimation of the real value of changes in $[K]_e$. The local changes of $[K]_e$ in response to single shock or adequate stimulation may therefore substantially exceed the changes established by K-ISMs (i.e. 0·5–1 mmol.l^{-1}).

The decrease of presynaptic spike amplitude which can lead to presynaptic inhibition would require a greater $\Delta[K]_e$, higher than 4 mmol.l^{-1}. On the other hand, K^+ accumulation may significantly influence the excitability of neurones as well as that of intraspinal terminals, even in the range of small $\Delta[K]_e$, 0·5–4·0 mmol.l^{-1}. Instead of any 'direct' effects of K^+ accumulation, shifts in $[K]_e$ may alter both

$[Ca]_e$ and $[Ca]_i$ and these should have a strong influence on the release of transmitters and lead to excitability changes. Besides K^+ accumulation may modulate spinal cord transmission by modifying glial cell function, by stimulation of oxygen consumption and by a variety of other biochemical processes and rate of protein synthesis. These and the remaining possible actions of K^+ persisting in intercellular space after previous activity deserve further attention.

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